Environmental Health Perspectives
Vol. 18, pp. 141-146, 1976

Studies in Adaption to Ambient Oxidant Air Pollution: Effects of Ozone Exposure in Los Angeles Residents vs. New Arrivals

by Jack D. Hackney,* William S. Linn,* Ramon D. Buckley,* and Helen J. Hislop†

To test the hypothesis that adaptation protecting against acute effects of ambient ozone (O_3) exposures develops in Los Angeles residents, human volunteers were exposed to 0.4 ppm O_3 under conditions simulating ambient pollution exposures. Blood biochemical, pulmonary physiological, and clinical responses were assessed. Los Angeles residents ($N = 6$) showed only minimal clinical or physiological response to O_3, while new arrivals ($N = 9$) showed significant losses in pulmonary function and a tendency toward increased symptoms. Most biochemical responses did not differ significantly between residents and new arrivals. These results agree with others in suggesting that exposures to elevated ambient concentrations of O_3 produce adaptation in at least some residents of photochemical pollution areas. The underlying mechanisms and long-term consequences of such adaptation are unknown.

Introduction

Development of tolerance to ozone (O_3) and other irritant gases in experimental animals was first described by Stokinger and co-workers approximately 20 years ago (*1*) and has been studied extensively since. The subject has been reviewed by Fairchild (*2*) and Morrow (*3*). Salient features of animal tolerance include the following. Pretreatment with a relatively low O_3 dose will prevent death or severe lung injury which would otherwise occur with a higher dose. This tolerance gradually disappears after cessation of O_3 exposure. Cross tolerance exists among O_3 and other irritant gases, including some which, like O_3, are powerful oxidizing agents and others which are not. Tolerance does not prevent the development of chronic lung lesions following repeated exposures. Tolerance results in decreased edema formation in response to O_3 challenge, but no diminution of cytotoxic effects of O_3 is observable (*4*). The biological mechanisms responsible for tolerance are largely unknown.

The observation that animals can respond to a toxic inhalation challenge in a manner which prevents some of the short-term adverse effects of further exposures suggests the possibility that an analogous response might occur in humans exposed to community air pollution. We use the term "adaptation" to describe this hypothetical response in humans, since the doses of toxicants being considered are much less than in animal "tolerance" studies, and since responses are less severe and perhaps depend on different biological phenomena. Metropolitan Los Angeles experi-

*Environmental Health Service, Rancho Los Amigos Hospital, 7601 East Imperial Highway, Downey, Calif. 90242.
†USC School of Medicine, Physical Therapy Department, 12933 Erickson Avenue, Downey, Calif. 90242.

ences uncommonly high ambient levels of O_3 and other oxidants during photochemical smog episodes; thus residents of this area constitute an attractive group in which to investigate the possibility of adaptation. That Los Angeles residents suffer less deleterious effects of ambient exposures than visitors to the area has been previously suggested (*5*), but the hypothesis has never been tested extensively.

Previous work in our laboratory (*6,7*) showed that some healthy Los Angeles residents develop respiratory symptoms and function changes when exposed to O_3 concentrations of 0.37-0.50 ppm—less than maximum ambient concentrations in the area. Similar studies in Canadians not frequently exposed to ambient oxidants (*8,9*) appeared to show a greater mean effect of a given dose, suggesting that responses in Los Angeles residents might have been reduced by adaptation. Methodological differences between studies might have explained the apparently different response, however. To test this possibility, a cooperative investigation was undertaken to compare experimental methods and responses of a small sample of subjects to 0.4 ppm O_3 (*10*). The results reproduced to a great extent the previous finding of less reactivity in Los Angeles residents as compared to Canadians and failed to reveal any methodological factors which could account for this difference. The hypothesis of adaptation was thus supported. To test the hypothesis more rigorously, the present study was undertaken in order to compare the effects of 0.4 ppm O_3 in somewhat larger and more carefully matched groups of Los Angeles residents and nonresidents.

Methods

The null hypothesis tested was as follows. Healthy Los Angeles residents (three years or more in area) and new arrivals (five days or less in area) will not differ in mean clinical, physiological or biochemical response to 0.4 ppm O_3 exposure under conditions simulating ambient pollution episodes. Rejection of the null hypothesis with new arrivals showing significantly greater mean response would be the necessary result if the hypothesis of O_3 adaptation in residents were to be supported.

The exposure facility and basic experimental design have been described in detail previously (*11*). Volunteer subjects were studied on two successive days. The first day's exposure was to purified air only; the second day's exposure was to 0.40 ppm O_3 in purified air. Exposures lasted 2 hr 15 min. During the first 2 hr, each subject exercised at a workload sufficient to increase minute volume to approximately twice the resting level (150-200 kg-m/min) for 15 min in every half hour. During the last 15 min pulmonary function tests were performed; these included forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), maximum midexpiratory flow rate (MMF), total respiratory resistance by forced oscillation (R_t), and indices of the single-breath nitrogen test: closing volume as a percent of vital capacity (CV/VC), and slope of the alveolar plateau (ΔN_2). Each subject's test results were expressed as control values (those obtained after purified-air exposure) and as O_3 responses (differences between post-O_3 exposure and control values). Subjects' symptoms during and following exposure were recorded and scored semiquantitatively according to severity and duration using a standard interview questionnaire administered by the project medical officer. The symptom response to O_3 was expressed as the difference in symptom score between O_3 exposure and control days. Venous blood samples were drawn immediately following exposure, and erythrocyte (RBC) and serum analyses were performed to detect changes expected to result from an oxidant challenge, as described previously (*12*).

Paired statistical tests with each subject serving as his own control were applied to detect differences between control and O_3 conditions for the resident group and for the new-arrival group. Unpaired tests were applied to compare between groups. For physiological measures, only O_3 responses were compared between groups, as control values were expected to depend mostly on body size and not on adaptation. For biochemical measures, control values could have differed between groups as a consequence of adaptation, therefore both control values and O_3 responses were compared statistically. In addition to the commonly employed *t* tests, analogous nonparametric tests—the Wilcoxon signed-rank test for paired analyses and the Mann-Whitney U test for between-group analyses—were applied to the pulmonary function data. The nonparametric tests were expected to be possibly more powerful in analyzing these data since the data were expected to be skewed, whereas *t* tests require a normal distribution for greatest reliability. Skewness is inherent in data of this nature since there is considerable variability between individuals in reactivity to exposure, and since

function measures remain similar to control values in relatively unreactive subjects but deviate from control values in only one direction in more reactive subjects. Symptom data, which were not rigorously quantitative and not necessarily expected to show a normal distribution even under control conditions, were analyzed only with the nonparametric tests.

Subjects were recruited within the incoming 1975 class of the USC School of Physical Therapy. Fifteen of a possible 44 individuals volunteered to be studied; six of these were residents of metropolitan Los Angeles and nine were nonresidents. Studies were conducted during September, i.e., late in the summer smog season when residents should have had ample time to develop adaptation. Nonresidents were studied within five days of their arrival in Los Angeles; they were instructed to minimize intercurrent ambient oxidant exposures by remaining in coastal areas of metropolitan Los Angeles and/or remaining indoors and at rest during peak oxidant hours.

Individual subject characteristics are given in Table 1. Since the nonresidents included two males, while the residents were all female, the possible effect of sex differences on the overall results was examined. The males' data were compared individually with the female nonresidents' for the three measures which showed significant ($p < 0.05$) group differences. Both males' values fell within the females' range, except that one male had the largest control and post-exposure FEV_1. When statistical analyses were repeated excluding the males' data, mean group responses were actually larger than when the males were included; however, due to the reduction in sample size the level of significance of the group differences decreased—$0.05 < p < 0.10$ with males excluded. Overall, no evidence was found that sex differences affected the results; this was also the case in the previous study (*10*).

Results

Individual physiological and clinical responses are given qualitatively in Table 1, and group mean physiological and symptom measures are summarized in Table 2. The residents as a group showed no significant O_3 responses except for slight decrease in ΔN_2. Increases in ΔN_2 are normally expected in chronic pulmonary dysfunction and in acute responses to O_3 exposure (*6*). Decreased values represent increased uniformity of ventilation distribution and thus could be considered an improvement in function. On the other hand, more uniform distribution could be the result of adverse physiological changes, such as complete "closure" of a few small airways previously only partially obstructed. Nonresidents

Table 1. Individual subject characteristics.

I.D. No.	Sex	Age, yr.	Ht., in.	Wt., lb.	Smoking	Years in Los Angeles area	O_3 response [a]
Los Angeles residents							
52	F	22	69	158	current	18	–
59	F	25	68	118	–	3	P
60	F	25	68	118	former	18	S
65	F	21	67	138	–	10	P
66	F	25	63	94	–	3	–
69	F	22	65	125	–	14	–
Nonresidents (new arrivals)							
47	F	22	68	140	–	–	P,S
49	M	22	71	160	–	–	P,S
50	F	21	66	115	–	b	–
51	F	21	62	125	–	–	P,S
53	F	22	73	155	–	–	–
55	F	22	68	125	–	–	S
56	F	23	62	120	–	–	P,S
57	F	21	64	121	–	–	P
58	M	24	72	170	current	–	P

[a] P = physiological response—significant ($p<0.05$) loss in FVC and/or FEV_1 with 0_3 exposure relative to control, determined by t test, three measurements under each condition. S = symptom response—increase in symptom score of ≥ 4 units (arbitrary definition of "clinically significant" response).

[b] Spent previous summer in area.

Table 2. Comparative pulmonary function and symptom measures: control values with O_3 exposure[a]

	Residents	New arrivals	Intergroup comparison	
			t	U
Control FVC, l	4.01 ± 0.40	4.57 ± 0.89		
FVC change, l	−0.093 ± 0.155 [b]	−0.164 ± 0.202 [c]	0.72 [b]	20 [b]
Control FEV_1, l	3.49 ± 0.22	3.84 ± 0.49		
FEV_1 change, l	−0.018 ± 0.098 [b]	−0.171 ± 0.174 [c]	1.93 [b]	9($p<0.05$)
Control MMF, l./sec	4.06 ± 0.70	4.23 ± 0.86		
MMF change, l./sec	+0.175 ± 0.336 [b]	−0.252 ± 0.320 [c]	2.48($p<0.05$)	9.5($p<0.05$)
Control CV/VC %	7.6 ± 5.5	6.8 ± 5.8		
CV/VC change, %	+0.4 ± 2.8 [b]	+0.5 ± 3.2 [b]	0.12 [b]	24 [b]
Control ΔN_2, % N_2/l	0.95 ± 0.15	0.93 ± 0.23		
ΔN_2 change, % N_2/l	−0.117 ± 0.094 [d]	−0.050 ± 0.206 [b]	0.73 [b]	21.5 [b]
Control R_n, cm H_2O/(l./sec)	4.02 ± 0.99	3.25 ± 0.90		
R_n, change, cm H_2O/(l./sec)	+0.13 ± 0.98 [b]	+0.20 ± 0.45 [b]	0.18 [b]	17 [b]
Control symptom score	4.9 ± 5.1	3.6 ± 3.5		
Symptom score change	+0.2 ± 5.5 [b]	+2.7 ± 4.8 [b]	—	19 [b]

[a] Means ± S.D.
[b] Not significant.
[c] Significant decrement after exposure, $p<0.05$ by paired *t* test and by Wilcoxon signed-rank test.
[d] Change not significant by signed-rank test; apparent "improvement" after exposure according to paired *t* test ($p<0.05$).

showed a smaller, nonsignificant decrease in ΔN_2, but showed significant O_3 responses in FVC, FEV_1, and MMF. The MMF response was significantly more severe than in the residents according to the intergroup comparison, but the FVC responses did not differ significantly between the groups. The FEV_1 loss was significantly more severe in nonresidents than in residents according to the U test ($p = 0.03$), but not according to the *t* test ($p = 0.06$). Since the distributions of FEV_1 responses appear skewed (Fig. 1), the results of the U test may be more reliable. Neither group showed significant responses of CV/VC, R_n, or symptom score, but the nonresidents showed a trend toward increased symptom score with O_3 exposure.

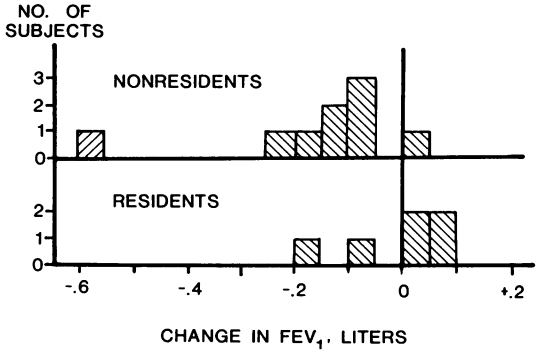

FIGURE 1. Histograms of O_3 responses in FEV_1 (change between post-exposure and control measurements) for nonresidents and residents. Number of subjects showing a given response (within a 0.05-l. interval) vs. magnitude of response.

Group mean biochemical measurements and significant changes related to exposure are summarized in Table 3. None of the analyses showed significant differences in control values between residents and new arrivals, although residents showed trends toward less fragility of RBCs as determined by hydrogen peroxide challenge, and higher serum concentrations of Vitamin E. Both groups showed O_3 responses generally similar to those seen previously (*12*): increased RBC fragility, reduced RBC acetylcholinesterase activity, and tendencies toward increased activity of pentose pathway enzymes (which would tend to protect against excessive oxidation of cellular components). Lactate dehydrogenase (LDH) activity was the only biochemical measure to show a significant difference between groups in response to O_3. New arrivals showed the expected increase in LDH activity, while residents showed a decrease, in contrast to previous findings (*12*). The biological significance of this observation, if indeed it represents other than a chance occurrence, is unclear.

Discussion

These results support the hypothesis of adaptation to O_3 in Los Angeles residents. Statistical differences found between residents and new arrivals are relatively small, as should be expected given the unavoidably small sample sizes and the

Table 3. Comparative biochemical measures: control values and change with O_3 exposure.[a]

		Residents	Intergroup comparison[b] New arrivals	t
RBC fragility,	Control	23.0 ± 15.2	31.4 ± 8.0	1.39[c]
% hemolysis in H_2O_2	Change	+6.2 ± 5.5[d]	+3.1 ± 3.4[d]	1.35[c]
RBC acetylcholinesterase,	Control	17.5 ± 1.8	18.8 ± 1.9	1.31[c]
mmole/ml/min	Change	−0.6 ± 0.6[e]	−0.8 ± 0.5[f]	0.83[c]
RBC glutathione,	Control	33.3 ± 6.2	35.3 ± 5.0	0.67[c]
mg %	Change	−1.6 ± 1.9[c]	−2.0 ± 3.0[c]	0.28[c]
RBC 2,3-diphospho-	Control	14.9 ± 1.6	14.7 ± 4.1	0.12[c]
glycerate, μmole/g Hb	Change	−1.7 ± 0.7[f]	+1.0 ± 3.3[c]	0.50[c]
RBC glucose-6-phosphate	Control	5.22 ± 1.14	5.05 ± 0.86	0.33[c]
dehydrogenase, U/g Hb/min	Change	+0.23 ± 0.43[c]	+0.21 ± 0.32[c]	0.09[c]
RBC lactate dehydrogenase,	Control	107 ± 16	112 ± 15	0.59[c]
U/g Hb/min	Change	−6.6 ± 12.4[c]	+8.9 ±9.5[f]	2.74[g]
RBC glutathione peroxidase,	Control	8.6 ± 1.8	8.8 ± 1.7	0.23[c]
U/ml/min	Change	+0.8 ± 1.1[c]	+0.3 ±0.8[c]	1.11[c]
Serum Vitamin E,	Control	2.77 ± 0.79	2.64 ± 0.38	0.44[c]
mg %	Change	+0.09 ± 0.15[c]	+0.03 ± 0.14[c]	0.66[c]
Serum glutathione	Control	23.7 ± 4.2	22.6 ± 3.0	0.60[c]
reductase, mU/ml/min	Change	+1.5 ± 1.7[c]	+2.8 ±3.0[f]	0.98[c]

[a] Means ± S.D.
[b] Intergroup comparisons by U test gave same results as t test in every case, with respect to significance at 0.05 level.
[c] Not significant.
[d] Significant ($P<0.05$) change after exposure by t test; not significant ($0.05<P<0.10$) by signed-rank test.
[e] Significant ($P<0.05$) change after exposure by signed-rank test; not significant ($0.05<p<0.10$) by t test.
[f] Significant ($p<0.05$) change after exposure by t test and by signed-rank test.
[g] Significant difference between groups ($p<0.05$).

typically large individual variability in O_3 responses. Controlled-exposure studies cannot be done on a large enough scale to conclusively establish differenes in response between populations, but the essetial agreement of present and previous results in small-scale studies considerably strengthens the case for the existence of such differences. Various factors unrelated to inherent adaptive biological reponses could explain these results—selective migration or diet, for example (*10*). No such factor has yet been identified, leaving adaptation as the most plausible explanation for the experimental observations. No biochemical index of the adapted state has yet been found in animals or in man, nor are the physiological and biochemical mechanisms of O_3 toxicity well understood. Further investigations in these areas will be necessary before the biological mechanisms of the adaptive response (if it exists) can be elucidated. Of particular interest is the possibility that adaptive mechanisms may be inoperative in certain individuals, who might then be at increased risk of developing chronic respiratory disease.

The phenomenon of adaptation may ultimately, but should not presently, be taken into account in setting ambient or occupational air-quality standards. By analogy with animal studies, it appears that human adaptation to acute O_3 effects might not protect against the possible development of chronic lung damage after many exposures. Unless this possibility and the possibility of failure of adaptation are conclusively ruled out, air quality standards should continue to be set to protect the susceptible, least well-adapted individuals in the exposed population.

From the Specialized Center of Research in Environmental Lung Disease (SCOR), National Heart and Lung Institute, Grant No. HL-15098-05.

This paper was presented in part at the Conference on Recent Developments in Toxicity of Environmental Oxidants, National Institute of Environmental Health Sciences, Bethesda, Maryland, March 4-5, 1976.

REFERENCES

1. Stokinger, H. E., Wagner, W. D., and Wright, P. G. Studies on ozone toxicity. I. Potentiating effect of exercise and tolerance development. Arch. Ind. Health 14: 158 (1956).
2. Fairchild, E. J. Tolerance mechanisms. Determinants of lung response to injurious agents. Arch. Environ. Health 14: 111 (1967).
3. Morrow, P. E. Adaptations of the respiratory tract to air pollutants. Arch. Environ. Health 14: 127 (1967).

4. Gardner, D. E., et al. Role of tolerance in pulmonary defense mechanisms. Arch. Environ. Health 25: 432 (1972).
5. Falk, H. L. Chemical definitions of inhalation hazards. In: Inhalation Carcinogenesis: AEC Symposium Series No. 18. Division of Technical Information, U.S. Atomic Energy Commission, Oak Ridge, Tenn., 1970.
6. Hackney, J. D., et al. Experimental studies on human health effects of air pollutants. II. Four-hour exposure to ozone alone and in combination with other pollutant gases. Arch. Environ. Health 30: 379 (1975).
7. Hackney, J. D., et al. Experimental studies on human health effects of air pollutants. III. Two-hour exposure to ozone alone and in combination with other pollutant gases. Arch. Environ. Health 30: 385 (1975).
8. Bates, D. V., et al. Short-term effects of ozone on the lung. J. Appl. Physiol. 32: 176 (1972).
9. Hazucha, M., et al. Pulmonary function in man after short-term exposure to ozone. Arch. Environ. Health 27: 183 (1973).
10. Hackney, J. D., et al. Health effects of ozone exposure in Canadians vs. Southern Californians: Evidence for adaptation? Arch. Environ. Health, in press.
11. Hackney, J. D., et al. Experimental studies on health effects of air pollutants. I. Design considerations. Arch. Environ. Health 30: 373 (1975).
12. Buckley, R. D., et al. Ozone and human blood. Arch. Environ. Health 30: 40 (1975); Air Pollution Abstr. p. 110, Abstr. No. 50966, September, 1975.